The Pharma Innovation Journal 2018; 7(5): 682-687

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(5): 682-687

www.thepharmajournal.com
Received: 06-03-2018
Accepted: 07-04-2018

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Evaluation of gum from *Abelmoschus esculentus* leaves as a suspending agent in cotrimoxazole suspension formulation

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Abstract

Gums from the dried leaves of *Abelmoschus esculentus* was extracted and evaluated as a suspending agent in cotrimoxazole suspension then characterized based on water solubility, swelling index, hydration capacity, proximate and elemental analysis. Standard gums such as betonite, tragacanth and sodium CMC were used for comparison and the values of the swelling index obtained were 1.83, 1.25, 1.40 and 12.40 for *A. esculentus*, and respectively for other suspending agents while the hydration capacity were 3.70, 1.80, 3.80 and 9.46 respectively as in swelling index. Proximate analysis of the *A. esculenta* leaf gum shows presence of moisture (12.20), ash (19.70), carbohydrate (16.67), protein (48.13), lipid (1.80) and fiber (1.51). The metal analysis reveals the presence of Fe, Pb, Mg, Ca, Na and K with values of lead obtained (2.55mg/kg). Cotrimoxazole suspension was formulated with percentage compositions of 0.5, 1.0 and 2.0%w/v of the suspending agents and evaluated for properties as: rheology, viscosity, sedimentation volume, redispersibillity and pH. Sodium CMC was observed to having consistent superior activity over the other gums in all parameters evaluated while the *A. esculentus* gum depicted behavior comparable to bentonite and tragacanth.

Keywords: *Abelmoschus esculentus* leaves, gum, cotrimoxazole, tragacanth, bentonite sodium CMC, suspension

Introduction

Pharmaceutical excipients play an important role in various pharmaceutical formulation and recent trends demands for the use and substitution of synthetic materials with natural ones.

The naturally derived excipients are attractive alternatives to synthetic ones because of their easy availability, biocompatibility, bio-degradable, low toxicity, environmental friendliness and low price compared to synthetic excipients [1].

In recent years plant gums have evoked tremendous interest due to their diverse pharmaceutical applications as binders in tablets, thickeners in liquid formulations, protective colloids in suspensions, gelling agents and bases in suppositories [2].

Pharmaceutical gums are termed as polysaccharide macro molecules that dissolve more or less upon contact with aqueous solvents forming a colloidal solution [3]. The gums have long been known for their medicinal value but recently newer uses has evolved especially in the preparation of cosmetics, textiles and in paint industries leading to increase in their demand [4].

Natural gums apart from being associated with various advantages as mentioned has some setbacks which includes: reduced viscosity on storage, easy microbial contamination and un controlled rate of hydration.

Abelmoschus esculentus, L

Fig 1: *Abelmoschus esculentus* plant

Description: This is an annual herb up to 5m tall, widely grown for its tender fruits and young leaves, easy to cultivate and grows well in both tropical and temperate zones including Africa, Asia, south Europe and America [5].

Mucilage obtained from the leaves is used as plasma replacement or blood volume expander, and reports has also revealed that an alcohol extract from Abelmeschus leaves can assist in eliminating oxygen free radicals, alleviate renal tubular intestinal diseases, improve renal functions and reduce proteinuria [6].

Pharmaceutical suspension is defined as preparations containing finely divided drug particle (the suspensoid), distributed uniformly throughout a vehicle in which the drug exhibits a minimum degree of solubility [7]. These suspensions are by nature thermodynamically unstable hence the approaches commonly adopted for the preparation of physically stable products, involves the use of structured vehicles such as suspending agent like polymers or polysaccharides notably, tragacanth, acacia, sodium carboxymethyl cellulose (NaCMC) [8].

Cotrimoxazole

This consists of two active chemical molecules such as: trimethoprim and sulphamethoxazole.

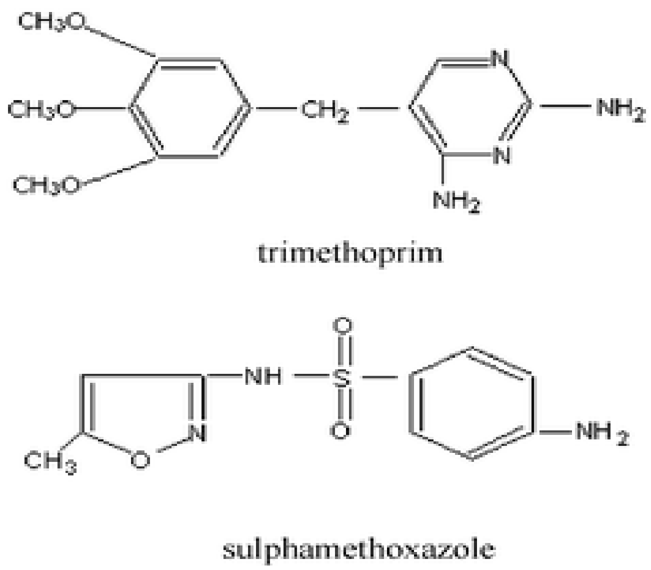

Fig 2: Structure of trimethoprim and sulphamethoxazole

The trimethoprim is an odorless white powder with a bitter taste, slightly soluble in water and some organic solvents like alcohol, chloroform but insoluble in ether. It is an antibacterial agent active against some range of organisms inhibited by the sulphanamides. And its mechanism of action includes the inhibition of dehydrofolate reductase [9].

Sulphamethoxazole is an odorless white or yellowish white crystalline powder with a slight taste and a bitter after taste. It is slightly soluble in water, alcohol and acetone. It is an anti-bacterial agent with a bacteriostatic activity and their chemical structure are seen to be similar to that of p-amino benzoic acid a substance essential for folic acid synthesis by the bacterial cell but unlike in mammalian cells is unable to absorb the preformed folate [10].

The purpose of this study is to source and evaluate for affordable and effective natural polymer especially from okra leaf to serve as a stabilizer and as an alternative to synthetic polymers in pharmaceutical formulations especially involving suspension drug delivery system.

Materials

Actone (kernel, China), sodium meta bisulphite, tragacanth (BDIH, England), sodium carboxymethyl cellulose (NaCMC) (Griffin and Geogy, England), bentonite (BDH, England), sodium chloride (May and Baker, England), Gelatin, pH meter (HANNA-H198107, USA), table centrifuge (PEC medical, USA), leaves of *Abelmoschus esculentus* obtained from Aluu in Port Harcourt, Rivers state, Nigeria.

Methods

In the extraction of mucilage from *Abelmoschus esculentus* leaves (Okra leaves, the method of Ordu and Chuckwu (2015) [11] was adopted and modified. A 200g of dried Okra leave was weighed and powdered then soaked in 1.5l of distilled water containing 0.1% sodium metabisulphite as an antioxidant. This was allowed to hydrate for 6hours then the mucilage extracted from the plant by squeezing through a muslin cloth to remove residues and obtain a translucent product. The extract was precipitated from the extraction fluid using acetone. The resulting precipitate was washed severally with same acetone to reduce moisture content of the gum. The precipitate was dried at 40°C in an oven and sieved through Bss 80 then percentage yield determined before storage in a desiccator.

Phytochemical examination

Preliminary tests namely: Ruthenium red, Molisch's and iodine tests were performed following standard procedures to confirm the nature of the gum obtained [12].

Physiochemical characterization of the gum

Solubility test: The gum was evaluated for solubility in water, ethanol, acetone and propylene glycol.

A 1.0g quantity of *A. esculentus* gum was weighed and placed in a clean test tube containing 10ml of water. The mixture was shaken vigorously and observed for solubility or dispersibility. This procedure was repeated using 10ml of other solvents (ethanol, acetone and propylene glycol).

Hydration and swelling capacity

These analyses were carried out according to the methods of Muazu *et al*, 2004 [13] and Bowen *et al*, 2005 [14] respectively for hydration and swelling capacity which were modified and adopted as described in the work of Ordu and Chukwu (2015) [11].

$$\text{Hydration capacity} = \frac{x}{Y} \quad \text{------------ (1)}$$

Where x= weight of moist sample after centrifugation
Y= weight of dry powder

$$\text{Swelling capacity} = \frac{Vs}{Vt} \quad \text{------------ (2)}$$

Where Vs = volume of sediment
Vt = tapped volume of powder

Proximate analysis of okra leaves gum

The proximate analysis involving percentage compositions of moisture, fiber, ash, lipid and carbohydrate of okra leaves gum were determined.

The moisture and ash were determined using weight difference; fiber content was estimated from the loss in weight of the crucible and its contents on ignition. Carbohydrate was determined when the sum of the percentage of moisture, ash, protein, lipid and fiber were subtracted from 100.

The nitrogen value which serves as precursor for protein content of a substance was determined by micro Kjeldalhi method described by Pearson (1976) [15] involving digestion, distillation and titration of the sample. The nitrogen value was converted to protein by multiplying with a factor of 6.25. Carbohydrate was determined by method of difference and all proximate values reported in percentage [16] [17].

Metal analysis: The following metals; Fe, Zn, pb, Ca, Mg, Na and k content of the okra leaves gum were analyzed following standard method involving use of atomic absorption spectrophotometer and according to the various absorbance wavelength of the designated metal ions.

Preparation of suspension

A 4.0g quantity of suphamethoxazole and 0.8g of trimethoprim was accurately weighed, triturated and levigated with 0.5ml of propylene glycol. The mixture was dispersed in 10ml of distilled water and in a mortar. Hydrated suspending agent was added gradually to the dispersed mixture and triturated properly. 3g of mannitol was dissolved in a beaker containing 10ml of water and then added to the mixture. The preparation was made up to 100ml with distilled water in a cylinder and stoppered. This procedure was adopted for preparations involving the various suspending agent which includes: okra gum, bentonite, tragacanth and sodium CMC in concentrations of 1.5%, 1% and 2% w/v. The degree of flocculation was determined, while flocculated suspensions were made using gelatin (1g) as flocculating agent and deflocculated suspensions made using sodium chloride (l.0g) as deflocculating agent [18].

Evaluation of prepared suspensions

The prepared suspensions were evaluated for pH, sedimentation volume [19] degree of flocculation [20] rheology [14] viscosity [21] and redispersibilityy [22].

Result

Percentage yield of gum from *A. esculenta* leaves = 9.6%.

Table 1: Phytochemical Analysis

Test	Observation	Inference
Ruthenium red test	Pink color	Gum present
Molisch's test	Violet color at junction of two layers	Carbohydrate present
Iodine test	Brown color	Starch absent

Physicochemical characterization

Table 2: Solubility Test and pH Determination

	Medium			
Suspending agent	**Water**	**Ethanol**	**Propylene glycol**	**Mean triplicate pH reading**
Okra leaves gum	soluble	insoluble	insoluble	7.33 ± 0.05
Bentonite	insoluble	insoluble	insoluble	8.60 ± 0.10
Tragacanth	soluble	insoluble	insoluble	6.20 ± 0.05
NaCMC	soluble	insoluble	insoluble	6.90 ± 0.05

Table 3: Hydration and Swelling capacity

Suspending agent	Hydration capacity	Swelling index
Okra gum	3.70 ± 0.8	1.83 ± 0.01
Bentonite	1.80 ± 0.1	1.25 ± 0.05
Tragacanth	3.80 ± 0.1	1.40 ± 0.06
NaCMC	9.46 ± 0.2	12.50 ± 0.05

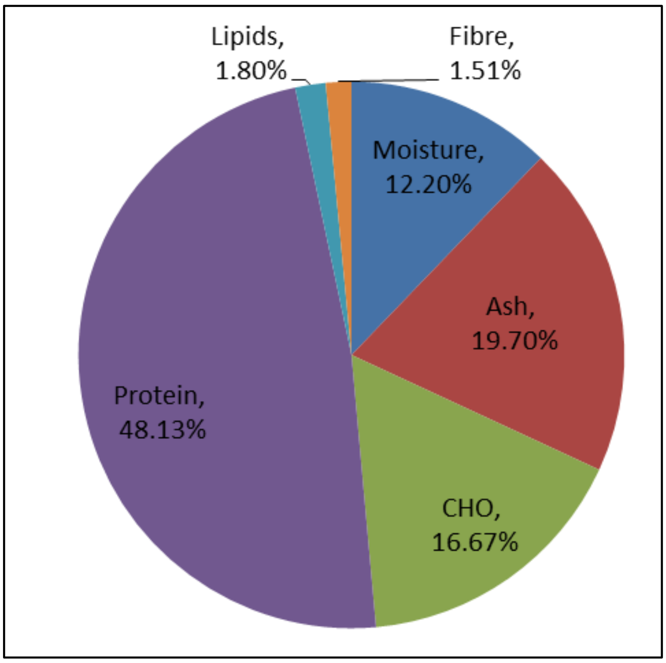

Fig 3: Proximate analysis of Gum from *Abelmoschus esculentus*

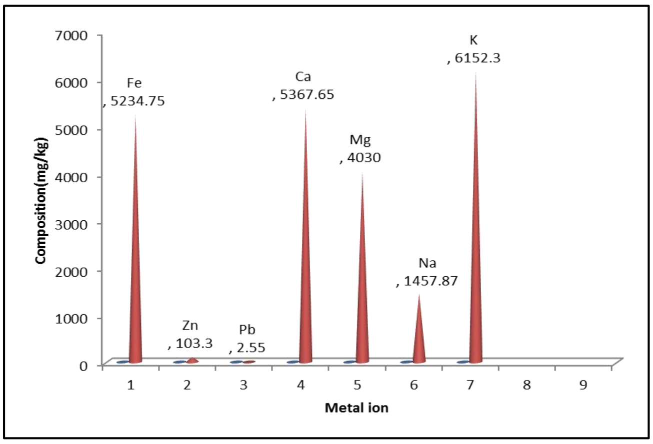

Fig 4: Metal analysis of Gum from *Abelmoschus esculentus*

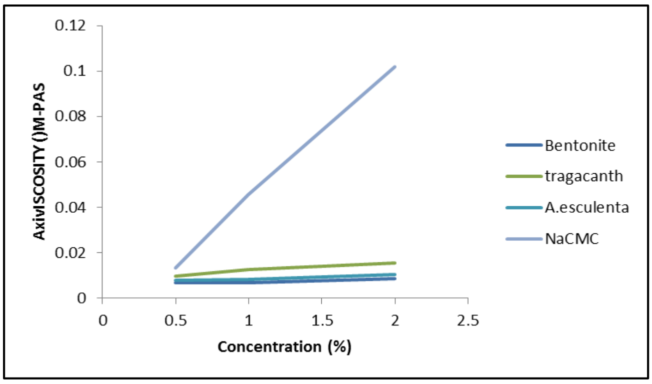

Fig 5: Viscosity profile of flocculated suspension using different concentration of suspending agent

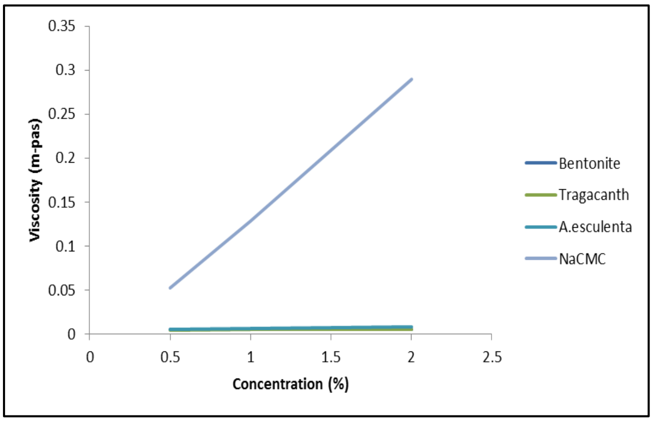

Fig 6: Viscosity profile of deflocculated suspension using varied suspending agent

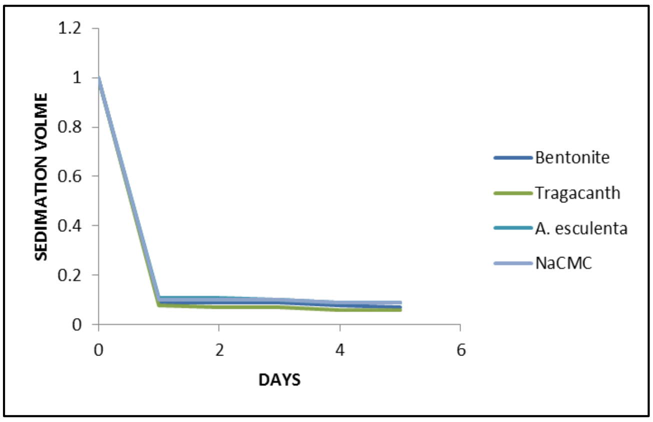

Fig 6: Sedimentation profile of flocculated suspension made with 0.5%w/v conc. of suspending agent

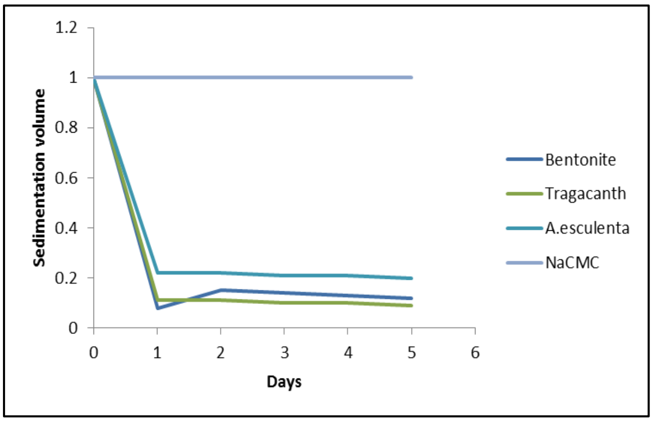

Fig 7: Sedimentation profile of flocculated suspension made with 2.0% w/v suspending agents

Discussion

The yield of gum obtained from *A. esculentus* leaves was 9.6%. The gum obtained was confirmed by subjection to preliminary phytochemical test using Ruthenium red, Molisch's test for presence of carbohydrate and absence of starch by iodine test.

Gum obtained from *A. esculentus* on physicochemical examination, is soluble in water but insoluble in organic solvents such as acetone, ethanol but sparingly soluble in propylene glycol.

Study of the water hydration capacity of the *A. esculentus* leaf gum in comparison with other standard suspending agents, shows NaCMC to have a value of 9.46 followed by Tragacanth 3.80, *A. esculentus* gum 3.70 then bentonite 1.30 depicting that the gum from *A. esculentus* leaf could be associated with low water retention capacity and only superior to bentonite but lower in activity to NaCMC and tragacanth.

Swelling index is an indication of the water absorption capacity of a substance and from the study involving the various suspending agents used NaCMC has a value of 12.50, followed by *A. esculentus* 1.83, tragacanth 1.40 and bentonite 1.25. This shows that *A. esculentus* leaf gum can impart some appreciable disintegrant property especially if used as an excipient in due to the appreciable water absorption capacity. especially in pharmaceutical solid dosage formulation but due to the low water retention ability as given in the hydration analysis might not be considered to have an effective binding property hence its suspending ability might be low. This fact is also supported by the result obtained on the rheology and viscosity of the suspension formulated with the gum.

Proximate analysis of *A, esculentus* leaf gum has percentage protein content to be highest (48.12%) while that of fiber is lowest at 1.50%. This result varies with that obtained by Adetunyi *et al* 2014 [23] with result of protein 20.30% and fiber 2.00%. This variation could be as a result of environmental factors where the leaves were grown and processed. The proximate analysis result also differs from that obtained from mucilage extracted from okra fruit having the following, protein content 7.31%, moisture content 9.37% and ash 4.81% [24].

The metal analysis as shown in fig.4 indicates presence of macro and micro elements with potassium taking the lead of the macro molecules (6152.30 mg/kg and sodium least at 1437.85mg/kg while for the micro molecules, iron occurred at the largest amount at 5234.75m/kg and zinc 103.30 mg/kg. The results therefore shows that despite the consideration of the extracted gum as excipient in pharmaceutical formulation its can also be useful as a food source in the human system function such as body cell repairs, energy enhancement and body resistance to infections. The amount of lead (Pb) found in the gum was 2.55mg/kg which is higher than the amount approved in infant formulations 0.01mg/kg [25], hence the use of the gum from *A. esculentus* leaves should be restricted in formulations (both pharmaceuticals and food) for administration to infants.

To evaluate the suspending property of the gum, co-trimoxazole suspension (trimethoprim/ sulphamethoxazole) was formulated in different batches containing suspending agents as: *A. esculentus* leaf gum, tragacanth, NaCMC and bentonite at various concentration range (0.5%, 1.0% and 2.0%) then flocculated and deflocculated.

Rheological studies of the prepared suspensions reveals the rate of flow to be inversely proportional to the concentration indicating that the viscosity increases with increase in concentration and this is more pronounced with NaCMC.

As in fig 5 and 6, viscosity of the flocculated suspension was found to be higher than the deflocculated and this can be ascribed to influence of the formation of aggregates known as flocs which is absent in deflocculated system where particles tend to exists mainly as discrete entities. The viscosity of the suspending agents was observed to increase in the order NaCMC > *A. esculentus* > bentonite > tragacanth.

Study of sedimentation rate reveals particles settling faster in flocculated than deflocculated systems producing a distinct boundary between the sediment and the supernatant especially for NaCMC (2% w/v) which remained suspended in both systems showing no clear boundary assuming the formulation to be in a state of flocculation equilibrium.

Sedimentation volume can have values ranging from < 1 to > 1. The more sedimentation volume approaches 1 the better the suspending property and in terms of the suspending behavior of the gums used, NaCMC > okra leaf gum > bentonite > Tragacanth. Though the sedimentation volume provides only a quantitative account of flocculation the degree of flocculation (β), is more useful a parameter and it is the ratio of ultimate sedimentation volume in flocculated and deflocculated systems.

Comparison of β values of various suspension formulations made with 2% w/v suspending agent shows NaCMC with the highest degree of flocculation (2.84) followed by bentonite (1.39), *A. esculentus* leaf gum (1.29) and Tragacanth (1.19).

The pH of the formulation made with *A. esculentus* leaf gum, gave values in the range of 6.5 to 4.5, indicating a slightly acidic nature and this result is similar to that obtained by Ravi *et al* 2009 [26] with value of 4.50 to 5.25. With this pH values the *A. esculentus* leaf gum could be assumed to be compatible in the formulation of basic drugs such as co-trimoxazole suspension

Since suspensions produce sediments on storage, it must be readily dispersible on slight agitation to ensure dosage uniformity. Study of the re dispersibility of suspensions shows that all the suspensions prepared especially the flocculated type were easily re dispersed including that made with 2% w/v content of NaCMC but for de flocculated systems re dispersion was difficult especially in that containing 2% w/v NaCMC.

Conclusion

Rheological studies of the formulations revealed the efficacy of the gums to follow the sequence NaCMC > bentonite > *A. esculentus* leaf gum > tragacanth.

The *A. esculentus* leaf gum could have appreciable disintegrant property especially if used as an excipient in Pharmaceutical solid dosage formulation but having low water retention ability it might not serve as an effective binding or suspending agent.

The extracted *A. esculenta* leaf gum though may be useful as an excipient in pharmaceutical tablets formulations, its usefulness can be extended to the human system function as it could help in body cell repairs, energy enhancement and body resistance to infections. The amount of lead(Pb) found in the okra leaves gum was higher than that approved in infant formulations, hence the use of the extracted okra leaf gum should be restricted in infant formulations.

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